

TESTING BIOMATERIALS FOR APPLICATION IN ARTIFICIAL ORGANS: IMPACT OF PROCEDURES, DONOR AND PATIENT PROPERTIES

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A b s t r a c t: Many factors can affect the characterisation of biomaterials during testing. These include drugs administered prior to testing and shear stress on blood cells induced by different blood flows and specific blood donor conditions. Some of the misconceptions in testing are described here and serve to indicate that a systems approach, and not only individual test parameters, is best when testing for biocompatibility.

"Methodology is everything and the devil is in the details", remarked Paul Simmons, the current president of the International Society for Stemcell Research, in an article in *Nature* magazine [1]. The article refers to current problems related to the reproducibility of data in stem cell research. Reproducibility in *in vitro* testing is also mandatory when selecting polymers for medical device applications. Many mechanical and physical engineers are surprised when they realise the enormous standard deviations (sometimes between 50 and 100%) of data found in biological or physiological investigations of biomaterials. The reasons for this are the complexity of physiological parameters such as the nature of blood originating from a variety of donors and hour-to-hour and day-to-day physiological differences. As a consequence, standardisation is a *conditio sine qua non* in biomaterial testing, and knowledge of possible pitfalls is absolutely necessary.

Therefore ISO 10993-4, Biological Evaluation of Medical Devices, Selection of Tests for Interaction With Blood, [2] provides a practical tool, including a decision tree for use in the selection of appropriate polymers for biomaterial applications. However, the interested reader finds in Section 3.1 of ISO 10993-4 the definition of blood-device interaction: "Any interaction between blood or any component of blood and a device, resulting in effects on blood, or on any organ or tissue, or on the device". A note added to this definition further clarifies: "Such effects may or *may not* have

clinically significant or undesirable consequences." This prompts one to ask if effects leading to undesirable consequences that are *not* clinically significant would be helpful to the polymer chemist.

This article provides some observations and examples of the misconceptions and pitfalls that exist in testing biomaterials for biocompatibility.

Key words: biomaterials, biocompatibility, artificial organs.

Importance of close simulation

The scientific literature offers a quantity of data on biocompatibility parameters. Polymers have been tested in relation to their capacity to cause coagulation, cell activation, inflammation and hypersensitivity (allergy) as well as their effects on the blood vasculature (Figure 1). It is still not yet clear which parameter best describes the final clinical situation. Conditions in testing should always simulate as closely as possible the clinical situation. Conditions such as the polymer surface area in contact with blood, blood flow in hollow fibre devices, and device sterilisation should be matched between the testing and the clinical situation. For example, blood trauma caused by repeated exposure to shear stress in a capillary tube of 1 mm inner diameter led to an irreversible stiffening of red cells, yet this effect was less pronounced in continuous shear conditions [3]. These observations may apply to blood recirculation experiments using a peristaltic pump and a low volume blood reservoir. In addition, miniaturised devices should always be assessed with a blood flow that is proportionally reduced to the actual surface area in contact with blood. By this means, mechanically induced shear forces remain in the same order of magnitude as those found in the original device.

Testing with healthy donors

Biocompatibility testing of polymers for application in medical devices is routinely performed with blood from healthy donors. However, medical devices are normally employed in patients suffering from a variety of diseases or during the administration of medicinal drugs that can interfere with polymer properties. It is questionable whether polymer testing with healthy blood represents the correct final clinical situation. Some examples originating from the application of biomaterials in haemodialysis are provided here to better explain this notion. This is a useful application to examine because, to date, the majority of publications on the biocompatibility of biomaterials in clinical

application deal with haemodialysis. This is no surprise. Chronic exposure of blood to foreign polymer surfaces takes place during haemodialysis treatment,

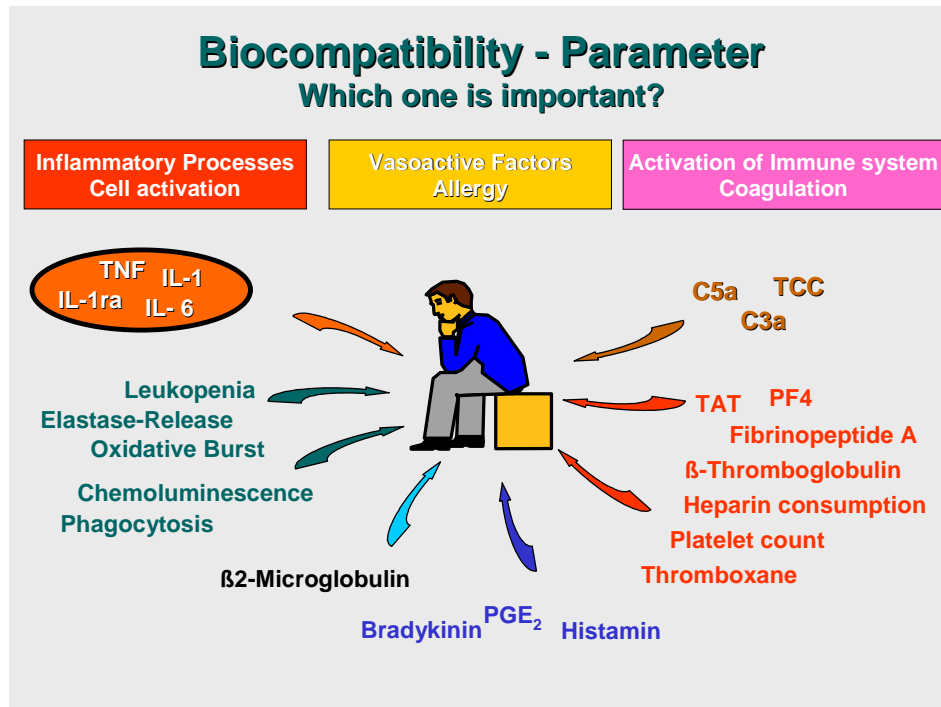


Figure 1 – Literature on biocompatibility parameters and their assessment would certainly fill a huge living-room. However, a clear-cut opinion in favour of one or the other parameter for a concise biomaterial characterisation is still lacking. A solution for this dilemma might be the application of a score model which incorporates a combination of several representative parameters

Слика 1 – Литература за биокompatбилниите параметри и нивното проценување несомнено ќе исполни огромна соба за живеење. Сепак, сèроѓо дефинирано мислење во полза на еден или друг параметар за концизна карактеризација на биоматеријал сè уште недостигава. Решение за оваа недоумица може да биде примена на модел на бодирање кој ќе инкорпорира комбинација на неколку репрезентативни параметри

because patients are treated thrice weekly for the rest of their lives. Furthermore, polymers for haemodialysis are used for a variety of applications such as capillary membranes, dialyser housings, tubing systems and bags for infusion

solutions. Thus, they are the most widely used biomaterials in the world. Their increasing application in single use devices means that even more biomaterials will be used in haemodialysis in the near future.

Patients with uraemia or with kidney disease experience disorders of blood physiology, that is, their blood cells and blood composition are different from normal blood. White blood cells from uraemic patients show an increased oxidative metabolism [4], a decreased antioxidant capacity depending on the progress of the uraemia [5], a reduced capacity for phagocytosis [6], and an increased number of reticulated platelets [7], which may lead to disturbances in the coagulation behaviour of uraemic blood. In addition, the lipid composition of uraemic platelets differs from normal thrombocytes [8], which may lead to a change in platelet aggregation. The behaviour of platelets in biocompatibility testing is extremely important because these cells determine the initial events of the blood-clotting cascade. For example, metabolically inhibited platelets do not adhere to glass test tubes, whereas their passive adhesion to polypropylene tubes remains unchanged [9]. How could this conclusion be reached if biocompatibility testing is only performed using blood from healthy donors?

Experiments on extracorporeal cardiopulmonary bypasses are normally assessed with blood from a calf or a pig. Analyses of blood count in the animal model after a prolonged perfusion of up to 7 days [10] proved that species type, whether bovine or porcine, had an impact on the haematology profile. Absolute values of red cell count were found to be higher in the calf, and normalised values were higher in the pig. Leukoctye counts did not behave similarly.

Testing and medication

A neglected area in biomaterials testing is the previous or simultaneous administration of medicinal drugs. Drugs such as aspirin or angiotensin-converting-enzyme (ACE) inhibitor may interfere with results on biocompatibility parameters to the extent that a clear-cut conclusion on whether or not a material is biocompatible cannot be drawn. Two examples illustrate this notion. After the administration of aspirin, the enzyme cyclooxygenase I is blocked. As a consequence, the formation of prostaglandins from arachidonic acid derived from activated cell membranes is also blocked and the thromboxanes A₂ or B₂ are not formed (Figure 2). The analysis of aspirin effects on the formation of thromboxane by different biomaterials used in haemodialysis such as polymethylmethacrylate (PMMA) and cellulose showed that the formation of thromboxane was halved with PMMA and completely blocked with cellulose [11] when 1000 mg of aspirin had been administered to the blood donor (Figure 3).

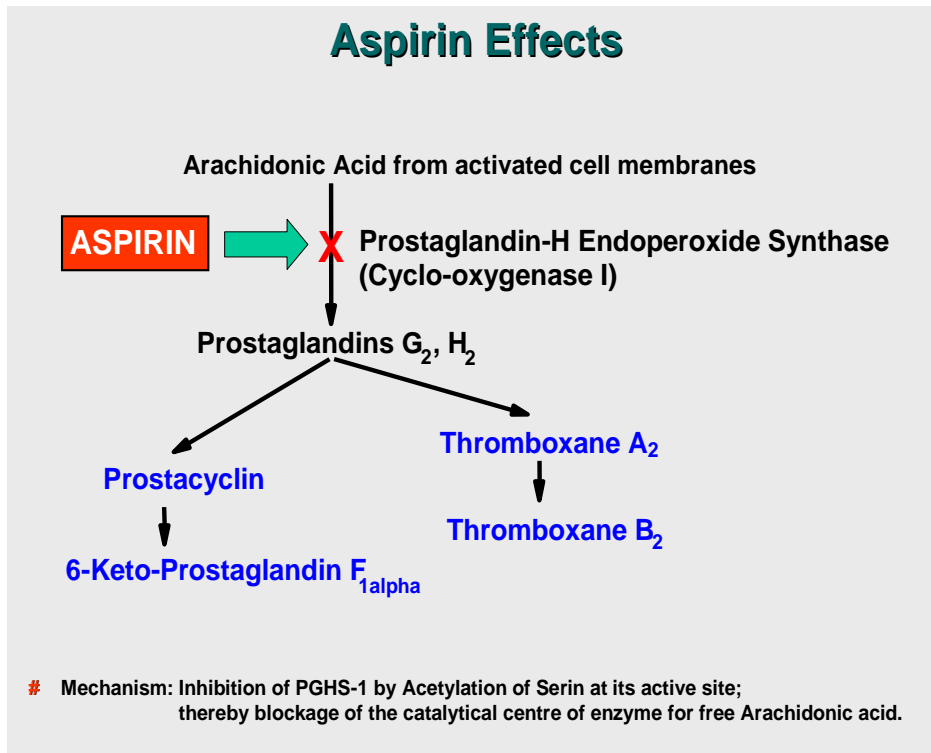


Figure 2 – Aspirin blocks the action of cyclooxygenase I on arachidonic acid from activated cell membranes. As a result, thromboxane and prostacyclin are not formed. Aspirin administration to blood donors, therefore, affects biocompatibility assessment of biomaterials

Слика 2 – Аспиринот го блокира дејствието на циклооксигеназаа на арахидонската киселина на активираниот клеточна мембрана. Како резултат на тоа тромбоксанот и простациклинот не се создаваат. Како последица на тоа, давање на аспирин на донорите на крв влијае на проценката на биокompatibilitетот на биоматеријалиот

A second example relates to the behaviour of biomaterials bearing a defined negative charge density, for example, the blend between polyacrylonitrile and methallylsulphonate employed as a polymer for capillary membranes for haemodialysis. Negative surface charges are able to stimulate the contact phase of coagulation, depending on charge density. As a consequence, a

cascade is initiated. This includes the formation of bradykinin, which is a nonapeptide that is able to down-regulate blood pressure through prostaglandin formation (Figure 4). It is fortunate that in the healthy human, the half-life of bradykinin is extremely short because of the activity of ACE. However, when ACE inhibitors are administered for medical reasons, the degradation process of ACE is inhibited and down-regulation of blood pressure is observed. This effect is dose-dependent, as shown in a sheep model (Figure 5, [12]).

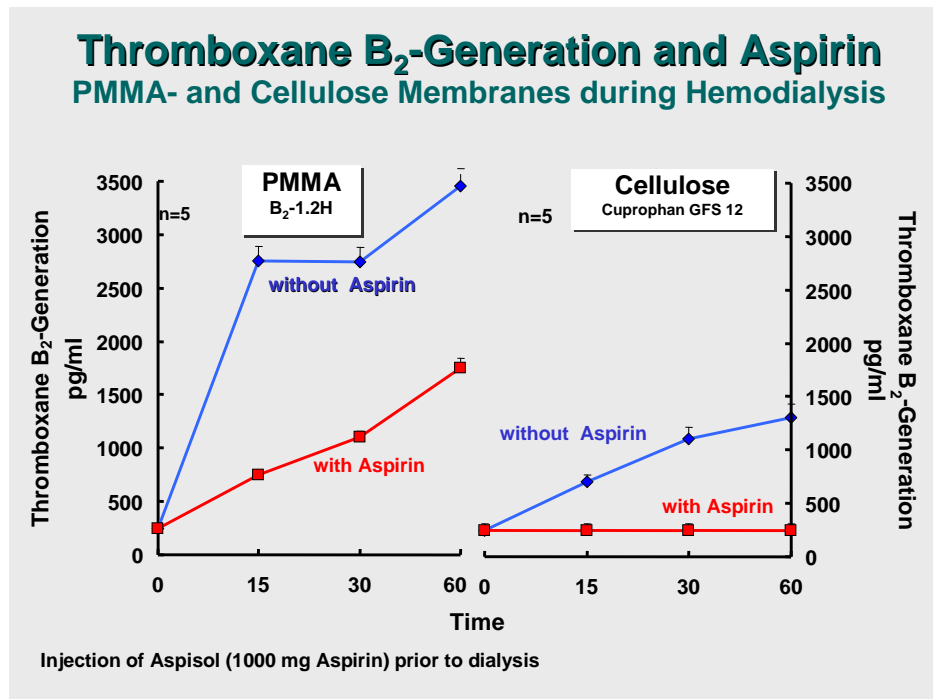


Figure 3 – Thromboxane formation by means of blood/material Interaction is affected by aspirin, whereby the level of reduction of thromboxane formation depends on the polymer type [11]

Слика 3 – Формирањето на тромбоксанот со помош на крв/материјал интеракцијата е афектирана од аспиринот, при што нивоето на намалување на формирањето на тромбоксанот зависи од типот на полимерот [11]

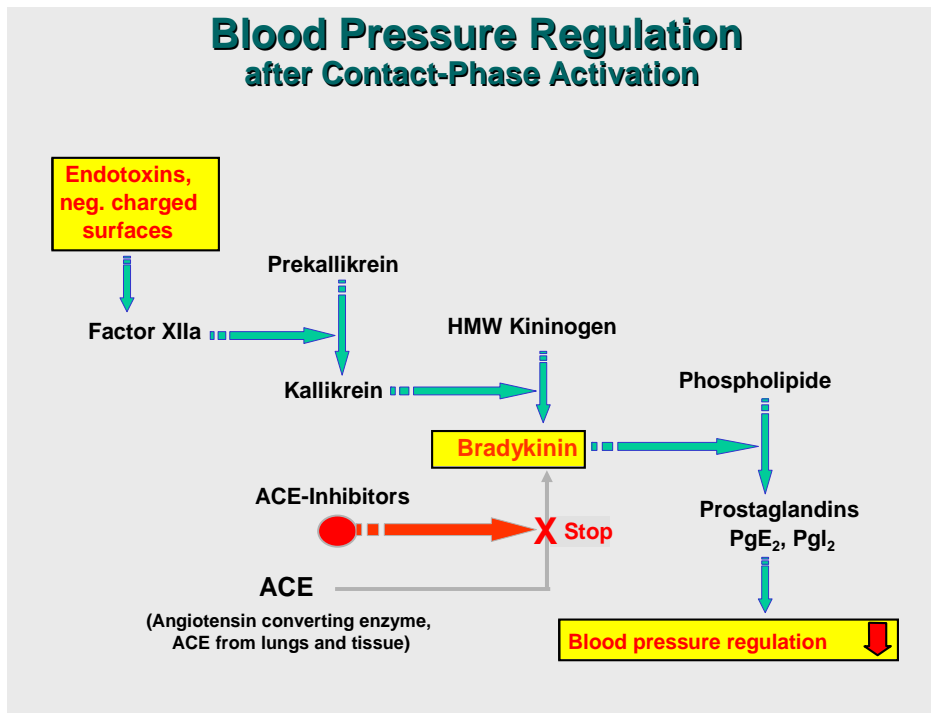


Figure 4 – Negatively charged biomaterials activate the contact phase of coagulation. The degree of activation depends on charge density. As a result, bradykinin is formed and blood pressure is down-regulated. Normally the half-life of bradykinin is short, due to the presence of angiotensin-converting enzyme-form tissue. In the presence of ACE-inhibitors, bradykinin degradation is inhibited and blood pressure down-regulation occurs

Слика 4 – Негајтивно набоеније биоматеријали ја активираат контактната фаза на коагулацијата. Степенот на активацијата зависи од густина на набојот. Како резултат на тоа се формира брадикининот и крвниот притисок е пониско регулиран. Нормално полуживото на брадикининот е краток како резултат на присуството на ткивниот ангиотензин конвертирачкиот ензим. Во присуство на АСЕ-инхибиторите разградбата на брадикининот е инхибирана и се јавува пониска регулација на крвниот притисок

This indicates that biocompatibility testing of biomaterials in the future should involve investigations on simultaneously administered medicinal drugs.

Old and new medicinal drugs may express synergistic effects between polymer composition and physiological cascades of the body, which can ultimately lead to adverse clinical events.

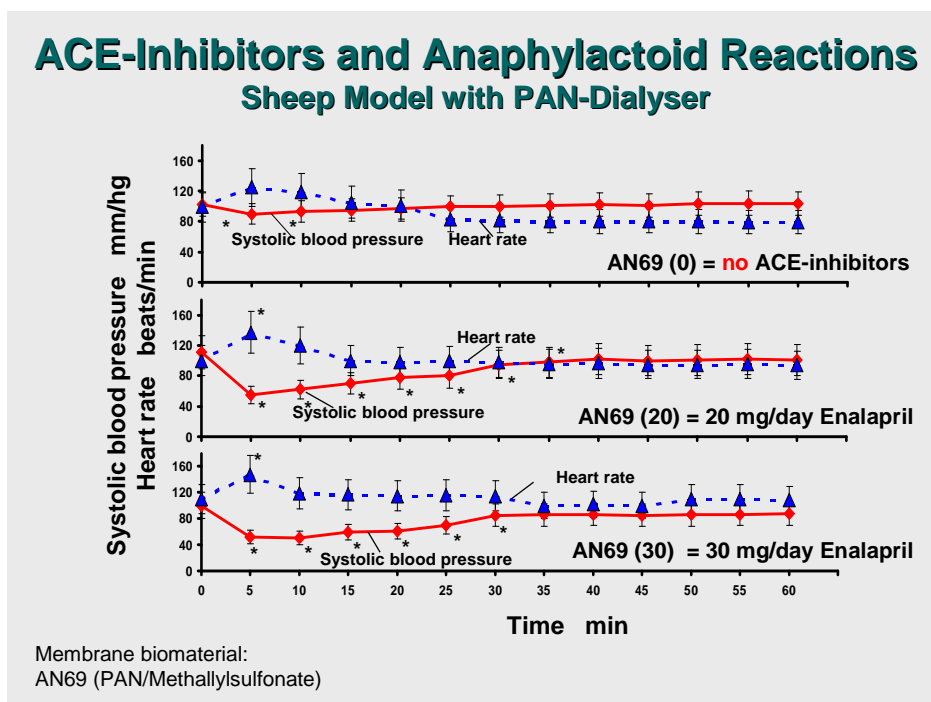


Figure 5 – Down-regulation of systolic blood pressure with negatively charged biomaterials depends on the presence of ACE-inhibitors in a dose-dependent manner, as shown in a sheep model. Blood pressure drop is partially counterbalanced here by an increased heart rate

Слика 5 – Понишка регулација на систолној крвен притисок со негативно наелектризиран биоматеријал зависи од присуството на ACE инхибитори во дозно зависен начин, како што е покажано на модел на овца. Падот на крвниот притисок е делумно контрабалансиран со порасот на фреквенцијата на работа на срцејто

Anticoagulants may also affect biocompatibility testing. Several physiological cascades such as complement and coagulation, as well as cell activation pathways, depend on the presence of Ca^{2+} or Mg^{2+} ions. The use of sodium citrate as an anticoagulant affects the release of the enzyme elastase from white blood cells, reduces complement activation and blocks the coagulation cascade, depending on the polymer under investigation [13].

Liquids for testing for extractables

"Blood is a very peculiar liquid," stated Mephisto to Faust in the drama *Faust*, by the German writer Johann Wolfgang von Goethe. Indeed, blood, which contains electrolytes, enzymes, lipids and proteins apart from water, is capable of extracting leachables from polymers or medical devices in a highly efficient manner. Consequently, biomaterial testing should always examine extraction capacity with the help of appropriate extraction media. One reason for the occurrence of extractables is a shift to broader molecular weight distribution during polymer synthesis (Figure 6). Polymer ageing adds to the source of extractables as well as the degradation of some polymers in a wet atmosphere or after some sterilisation procedures.

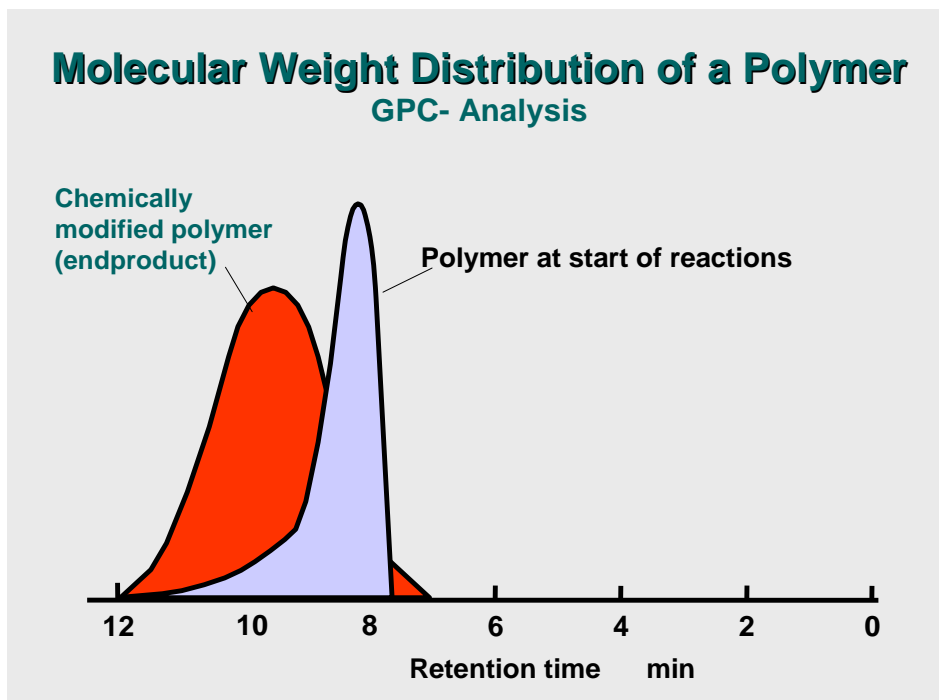


Figure 6 – The GPC analysis of polymer formation shows a broadening of the polymer's molecular weight distribution at the end of reaction. This effect may give rise to extractable material which may leach out in contact with blood

Слика 6 – GPC анализа на формирањето на полимерите покажа проширување на тежината на дистрибуцијата на молекулите на полимерот на крајот од реакцијата. Тој ефект може да даде повод за екстракцибилниот материјал кој може да испече во контакт со крвта

ISO 10993-12 prescribes those extraction media that should be used for the isolation of leachables [14]. Solvents, selected as extractants, "shall simulate the extraction which occurs during clinical use of the device and/or maximise the amount of extract." For a reliable extraction of all leachables three types of extractants should be used, and using all three allows the blood properties for extraction to be simulated effectively:

1. Polar media such as water, saline (0.9% NaCl) or culture media without serum derived from cell culture technologies;
2. Nonpolar media such as vegetable oil (cotton seed or sesame oil);
3. Mixtures of ethanol-water, polyethyleneglycol 4000 (PEG 4000), dimethyl-sulfoxide (DMSO), or culture media with serum.

Recently, severe adverse clinical events [15, 16] and even fatal incidences [17] originating from extractable material have been reported in the scientific literature. These data show that aged polymers [15], degraded polymers [16] or liquids used for device integrity tests during the manufacturing process [17] may give rise to extractable material. This can later be extracted from medical devices and accumulated in patients associated with adverse clinical events. In many of these cases analyses are extremely time-consuming because the correct extractant has not been applied in the first instance.

Recommendations

It has been shown that a series of factors may interfere with the biocompatibility performance of polymers. Among these are blood donor specificities, anticoagulants, physical conditions of blood flow and drugs administered to the blood donor. Consequently, a simple and clear-cut analysis of testing results is difficult and sometimes even unpredictable. Testing polymers and other biomaterials for their application in medical devices should always be performed as a systems approach taking into account a series of side-effects. The development of a score model [18] which summarises several impact factors at a time may be a solution.

REFERENCES

1. *Nature*, (2007).446: 485–486.
2. International Standard ISO 10993-4 Biological evaluation of medical devices. Part 4: selection of tests for interactions with blood. 2nd Edition. ISO, 2002, 10–15.

3. Lee S.S. *et al.* (2007): Strain Hardening of Red blood cells by accumulated cyclic suprphysiological stress. *Artif Organs*; 31: 80–86.
4. Paul J. *et al.* (1991): Influence of uremia on polymorphonuclear leukocytes oxidative metabolism in endstage renal disease and dialysed patients, *Nephron*; 57: 428–432.
5. Mimic-Oka J. *et al.* (1999): Alteration in plasma antioxidant capacity in various degrees of chronic renal failure, *Clin Nephrol*; 51: 233–241.
6. Alexiewicz J. *et al.* (1991): Impaired phagocytosis in dialysis patients: studies on mechanisms, *Am J Nephrol*; 11: 102–111.
7. Himmelfarb J. *et al.* (1997): Increased reticulated platelets in dialysis patients, *Kidney Int*; 51: 834–839.
8. Vecino A. *et al.* (1998): Lipid composition of platelets in patients with uremia, *Nephron*; 78: 271–273.
9. LaFayette N. *et al.* (2007): An *in vitro* method for assessing biomaterial-associated platelet activation, *ASAIO J*; 53: 159–162.
10. Mueller X. *et al.* (2001): Hemolysis and hematology profile during perfusion: inter-species comparison, *Int J Artif Organs*; 24: 89–94.
11. Vienken J. and Schäfer R. (1995): Acetyl-Salicylsäure (Aspirin) beeinflusst die Generation von Thromboxan durch Dialysemembranen. *Nieren & Hochdruckkrankheiten*, 24: 480.
12. Krieter D. *et al.* (1998): Anaphylactoid reactions during hemodialysis in sheep are ACE inhibitor dose-dependent and mediated by bradykinin. *Kidney Int*; 53: 1026–35.
13. Böhrler J. *et al.* (1993): Mediators of complement-independent granulocyte activation during haemodialysis: role of calcium, prostaglandins and leukotriens, *Nephrol Dial Transplant*; 8: 1359–1365.
14. ISO 10993-12 Biological Evaluation of Medical Devices, Part 12: Sample Preparation and Reference Materials, 2nd Edition. Corrected version 2003–06–01.
15. Hutter J. *et al.* (2000): Acute onset of decreased vision and hearing loss traced to hemodialysis treatment with aged dialysers, *JAMA*; 26: 2128–2134.
16. Averbukh Z. *et al.* (2001): Red eye syndrome: clinical and experimental experience in a new aspect of diffuse eosinophilic infiltration? *Artif Organs*; 25: 437–440.
17. Canaud B. *et al.* (2005): Pathochemical toxicity of perfluorocarbon, a liquid test performance fluid previously used in dialyser manufacturing, confirmed in an animal experiment. *J Am Soc Nephrol*; 16: 1819–1823.
18. Erlenkoetter A. *et al.* (in press 2008): Score model for the evaluation of dialysis membrane biocompatibility. *Artif Organs*.

Резиме

**ТЕСТИРАЊЕ НА БИОМАТЕРИЈАЛИ ЗА ПРИМЕНА
ВО ВЕШТАЧКИТЕ ОРГАНИ: ВЛИЈАНИЕТО НА ПОСТАПКИТЕ,
МОЖНОСТИТЕ НА ДОНОРИТЕ И ПАЦИЕНТИТЕ**

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Многу фактори можат да влијаат на карактеризацијата на биоматеријалите за време на тестирањето. Тоа вклучува лекови давани пред тестирањето и го дели стресот врз крвните ќелии предизвикан од различни крвни протоци и специфични состојби на крвта на донорот. Некои од погрешните сфаќања во тестирањето се опишани овде и служат да укажат дека системскиот приод, а не само поединечните параметри на тестот, е најдобар кога ја тестираме биокомпатибилноста.

„Методологијата е сè и ѓаволот е во подробностите“, забележал Пол Симон, сегашниот претседател на Меѓународното здружение за истражување на матични клетки, во текстот во списанието Nature [1]. Текстот се однесува на сегашните проблеми кои се однесуваат на можноста за репродуцирање на податоците во истражувањето на матичните клетки. Можноста за репродуцирање во ин витро тестирањето е исто задолжителна кога се избираат полимери кои се применуваат во медицинските уреди. Бројни машински инженери и физичари се изненадени кога ги согледуваат огромните стандардни девијации (понекогаш помеѓу 50 и 100 %) на податоците најдени при биолошките или физиолошките истражувања на биоматеријалите. Причините за тоа се во сложеноста на физиолошките параметри како што е природата на крвта што потекнува од различни донори и физиолошките разлики од час во час и од ден до ден. Како последица, стандардизацијата е *conditio sine qua non* во тестирањето на биоматеријалите и познавањето на можните замки е апсолутно потребно.

Затоа, ISO 10993-4, Биолошка евалуација на медицински уреди, селекција на тестови за интеракција со крв [2] претставува практично алатка, вклучувајќи стебло на одлуки за употреба во одбирање на погодни полимери за примена на биоматеријали. Сепак, заинтересираниот читател во делот 3.1 од ISO 10993-4 ќе најде дефиниција за интеракција на крвта и уредот: „Секоја интеракција меѓу крвта или која било компонента од крвта и уредот, причинува последици на крвта, или на било кој орган или ткиво, или на уредот“. Забелешката додадена на таа дефиниција понатаму разјаснува: „Такви ефекти можат или не можат да имаат клинички значајни или несакани последици.“ Тоа доведува до прашањето дали ефектите кои водат до несакани последици што не се клинички значајни ќе им бидат од помош на хемичарите за полимери.

Оваа статија дава некои опсервации и примери на погрешните претстави и замки што постојат при тестирање на биоматеријали за биокомпатибилност.

Клучни зборови: биоматеријали, биокомпатибилност, вештачки органи.

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